

Deng et al., Supplemental Discussion and Complete List of Genotypes

Characterization of intracellular Ca²⁺ oscillations in ISCs

To assess the relative requirement for extracellular Ca²⁺ and intracellularly stored Ca²⁺ for cytosolic Ca²⁺ oscillations, we recorded Ca²⁺ oscillations in intestines exposed to Ca²⁺ chelators and Ca²⁺ channel inhibitors. Cytosolic Ca²⁺ oscillations can be triggered by Ca²⁺ influx from the extracellular space through ligand- or voltage- gated ion channels, or by Ca²⁺ released from intracellular stores such as the ER through IP3 Receptors. G-protein coupled receptors (GPCRs) or receptor tyrosine kinases (RTKs) generate IP3 at the plasma membrane by activating phospholipases ¹⁻³. The extracellular Ca²⁺ chelator EGTA, the nonspecific Ca²⁺ channel inhibitors CdCl₂ and LaCl₃, and 2-APB (2-aminoethoxydiphenyl borate), an antagonist of IP₃ Receptors (which may, however, also inhibit SOCE channels)⁴, all inhibited Ca²⁺ oscillations (Extended Data Fig. 5b, c; to quantify Ca²⁺ oscillation parameters, spikes were quantified both manually as well as using automated peak detection, with similar results, Extended Data Fig. 5d). [Ca²⁺] oscillations in ISCs thus depend on both influx through plasma membrane Ca²⁺ channels as well as on Ca²⁺ release from intracellular stores through IP3 receptors, a notion supported by the fact that genetic knockdown of IP3 receptors also impairs Ca²⁺ oscillations (Fig. 3a).

Incubation with the L-type voltage gated Ca²⁺ channel (VGCC) inhibitor Isradipine did not affect Ca²⁺ oscillations in ISCs (Extended Data Fig. 5f). Isradipine paralyzes visceral muscles, and is used in most experiments reported here to limit gut movements.

SOCE-regulated Ca²⁺ dynamics

The observed effects of genetic perturbations shown in Fig. 3 and Extended Data Figs. 7, 8 and 9, and their interpretation are discussed in detail below:

Knock down of SERCA (using the ISC-specific combination of esg::Gal4 with Su(H)Gbe::Gal80), or incubation of fly intestines with thapsigargin, a SERCA inhibitor, is sufficient to inhibit Ca²⁺ oscillations, while strongly increasing [Ca²⁺] in ISCs (Fig. 3a, Extended Data Fig. 7a, and Supplemental Video 8). Knock down of STIM also significantly decreases Ca²⁺ oscillation frequency, without significantly affecting average intensity (Fig. 3a, Extended Data Fig. 7a, and Supplemental Video 9). This is consistent

with a critical role for SOCE in maintaining Ca^{2+} oscillations in ISCs, without significantly impacting Ca^{2+} amounts released from the ER. Knockdown of STIM also strongly suppresses the increase in cytosolic Ca^{2+} observed in SERCA loss of function conditions, confirming the requirement for SOCE in Ca^{2+} influx into the cell (Fig. 3a).

Knocking down the sole *Drosophila* IP3 receptor (IP3R) substantially reduces Ca^{2+} oscillation frequency and maintains low average cytosolic $[\text{Ca}^{2+}]$, while silencing the ryanodine receptor (RyR) homolog does not change the oscillation pattern (Fig. 3a; all the RNAi lines used in this study were either validated by qPCR or have been previously published; see Materials and Methods).

Co-overexpression of STIM and Orai, over-expression of IP3R, or knocking down PMCA reduces oscillation frequency and increased the average signal intensity in ISCs (Fig. 3a, Extended Data Fig. 7a), while silencing Voltage gated Ca^{2+} channels (VGCCs), potassium channels, or the NMDA receptor does not change the Ca^{2+} oscillation pattern (Extended Data Fig. 6f and data not shown).

Control of ISC proliferation by changes in cytosolic $[\text{Ca}^{2+}]$ and effects of SERCA knockdown on Notch signaling

When SERCA is knocked down (using the ISC/EB driver *esg*::Gal4, or the ISC-specific combination of *esg*::Gal4 with *Su(H)Gbe*::Gal80), or inhibited by Thapsigargin, ISCs undergo strong over-proliferation, resulting in increased mitotic figures, Dl+ cells, and epithelial overgrowth (Fig. 3b, c, Extended Data Fig. 7b, c; note that strong over-proliferation of ISCs often results in a moderate increase in Dl+ cells, see Bleomycin treatment in Extended Data Fig. 8d,e). Knocking down SERCA in EBs only, using *Su(H)Gbe*::GAL4, on the other hand, failed to induce proliferation (Extended Data Fig. 7b). Flies carrying the dominant-negative temperature-sensitive SERCA allele *Serca*^{Kum170}⁵ also showed a significant increase of mitotic cells in the intestine after a short term heat shock (Extended Data Fig. 7d; 5min, 42 °C for two consecutive days, this leads to long-term impairment of Ca^{2+} homeostasis in *Serca*^{Kum170} mutants), and *Serca*^{Kum170} mutant or *Serca*^{RNAi} expressing MARCM clones grew significantly faster than controls (Extended Data Fig. 7c, d). Similarly, decreasing Ca^{2+} efflux through the plasma membrane by silencing PMCA, or increasing Ca^{2+} influx into the cell by co-overexpression of *Stim* and *Orai*, or increasing Ca^{2+} release from the ER into the

cytoplasm by overexpressing IP3R, induced ISC proliferation, resulting in increased pH3 positive ISCs, epithelial overgrowth, and increased clone growth by MARCM analysis (Fig. 3b, Extended Data Fig. 7c, f).

The phenotypes observed in SERCA-deficient ISCs could be a consequence of ISC proliferation stimulated by elevated cytosolic $[Ca^{2+}]$ or of a lack of differentiation due to Notch misfolding, as had been proposed⁶. To distinguish between these possibilities, we assessed if perturbing STIM, and thus limiting Ca^{2+} entry into the cytoplasm, could rescue SERCA loss of function phenotypes. Loss of STIM did not impact ISC proliferation under homeostatic conditions (when ISC proliferation rates are very low anyway), but resulted in slightly reduced MARCM clone growth (Fig. 3b, c, Extended Data Fig. 7 c, e; using the null allele *Stim*⁴⁷). Silencing STIM in SERCA deficient ISCs, however, significantly reduced cytosolic $[Ca^{2+}]$ and ISC over-proliferation (Fig. 3 a-c; Extended Data Fig. 7 f).

These results suggest that the elevation of cytosolic $[Ca^{2+}]$ is critical for the acute proliferative response of SERCA deficient ISCs, and that SERCA-independent elevation of cytosolic $[Ca^{2+}]$ (knockdown of PMCA, over-expression of STIM and Orai, or of IP3R) is sufficient to promote ISC proliferation. However, characterization of N pathway activity and of N-mediated differentiation phenotypes also revealed that prolonged knockdown (7-14 days) of SERCA indeed inhibits N activity in the ISC lineage (as described in⁶; Extended Data Fig. 8a-f). Prolonged loss of SERCA results in loss of N-dependent Su(H),Gbe::lacZ reporter activity and in ‘tumors’ composed of Dl+ ISCs and Prospero+ EEs (Extended Data Fig. 8a-f). A contribution of N inhibition to the proliferative response of ISCs in SERCA loss of function conditions can thus not be excluded. These phenotypes were not observed, however, during short-term knockdown of SERCA (4 days, a time point at which strong induction of ISC proliferation is already observed; Fig. 3b, c), nor when cytosolic $[Ca^{2+}]$ and ISC proliferation were increased by knockdown of PMCA, or when ISC proliferation was increased by over-expression of CRTC (Extended Data Fig. 8a-f). MARCM clones further revealed that SERCA and PMCA deficient lineages are capable of generating fully differentiated Pdm1 expressing ECs, while N deficient lineages are not (Extended Data Fig. 8g, h).

Interestingly, loss of N itself leads to sustained elevated $[Ca^{2+}]$ in ISCs (Extended Data Fig. 10b), and increased proliferation of N-deficient ISCs requires Ca^{2+}/CaN signaling (Extended Data Fig. 8i, see below), indicating that Ca^{2+} signaling acts as a downstream mediator of ISC proliferation in N loss of function conditions. Artificially increasing N activity (by expression of N intracellular domain, N^{ICD}) failed to fully inhibit ISC proliferation in SERCA loss of function conditions (Extended Data Fig. 8j), further indicating an N independent regulation of proliferation in this context.

Overall, our results suggest that the proliferative effects of elevated $[Ca^{2+}]$ signaling can be decoupled from the effects of SERCA ablation on N activity. This interpretation is consistent with the increase in cytosolic $[Ca^{2+}]$ under a wide range of mitogenic conditions (including infection, nutrients, DNA damage, etc.), with the reversibility of the proliferative response (and the lack of N loss of function phenotypes) in these conditions, and with the fact that elevating cytosolic $[Ca^{2+}]$ independently of SERCA triggers ISC divisions without causing N loss of function phenotypes. However, loss of SERCA may perturb N activity mildly, contributing to the proliferative activation of ISCs without causing immediate differentiation phenotypes. Such an interaction would be interesting and needs to be evaluated using real-time and sensitive assessment of N activity.

References

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target in NOTCH1 mutated cancer. *Cancer Cell* **23**, 390-405,
doi:10.1016/j.ccr.2013.01.015 S1535-6108(13)00037-8 [pii] (2013).
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Complete List of Genotypes for Figures

Fig. 1b:

$w^{1118}/UAS::Dicer2^{OE}$; $esg::Gal4$, $UAS::YFP$; $tub::Gal80^{ts}$, $Su(H)Gbe::Gal80/+$
 $w^{1118}/UAS::Dicer2^{OE}$; $esg::Gal4$, $UAS::YFP$; $tub::Gal80^{ts}$, $Su(H)Gbe::Gal80/UAS::mGluR^{RNAi}$

Fig. 2d:

w^{1118} ; $esg::Gal4$, $UAS::mCherry$, $UAS::GCaMP3$; $tub::Gal80^{ts}$, $Su(H)Gbe::Gal80/+$
 w^{1118} ; $esg::Gal4$, $UAS::mCherry$, $UAS::GCaMP3$; $tub::Gal80^{ts}$,
 $Su(H)Gbe::Gal80/UAS::mGluR^{RNAi}$
 w^{1118} ; $esg::Gal4$, $UAS::mCherry$, $UAS::GCaMP3$; $tub::Gal80^{ts}$, $Su(H)Gbe::Gal80/UAS::GaQ^{RNAi}$

Fig. 2e:

w^{1118} ; $esg::Gal4$, $UAS::eYFP$, $tub::Gal80^{ts}$, $Su(H)Gbe::Gal80/+$
 w^{1118} ; $esg::Gal4$, $UAS::eYFP$, $tub::Gal80^{ts}$, $Su(H)Gbe::Gal80/UAS::PlcB^{RNAi}$
 w^{1118} ; $esg::Gal4$, $UAS::eYFP$, $tub::Gal80^{ts}$, $Su(H)Gbe::Gal80/UAS::GaQ^{RNAi}$

Fig. 3a:

w^{1118} ; $esg::Gal4$, $UAS::mCherry$, $UAS::GCaMP3$; $tub::Gal80^{ts}$, $Su(H)Gbe::Gal80/+$
 w^{1118} ; $esg::Gal4$, $UAS::mCherry$, $UAS::GCaMP3$; $tub::Gal80^{ts}$,
 $Su(H)Gbe::Gal80/UAS::Serca^{RNAi}$
 w^{1118} ; $esg::Gal4$, $UAS::mCherry$, $UAS::GCaMP3$; $tub::Gal80^{ts}$,
 $Su(H)Gbe::Gal80/UAS::Pmca^{RNAi}$
 w^{1118} ; $esg::Gal4$, $UAS::mCherry$, $UAS::GCaMP3$; $tub::Gal80^{ts}$,
 $Su(H)Gbe::Gal80/UAS::Stim^{RNAi}$
 w^{1118} ; $esg::Gal4$, $UAS::mCherry$, $UAS::GCaMP3$; $tub::Gal80^{ts}$, $Su(H)Gbe::Gal80/UAS::Stim^{RNAi}$,
 $UAS::Serca^{RNAi}$
 w^{1118} ; $esg::Gal4$, $UAS::mCherry$, $UAS::GCaMP3$; $tub::Gal80^{ts}$,
 $Su(H)Gbe::Gal80/UAS::IP3R^{RNAi}$
 w^{1118} ; $esg::Gal4$, $UAS::mCherry$, $UAS::GCaMP3$; $tub::Gal80^{ts}$,
 $Su(H)Gbe::Gal80/UAS::RyR^{RNAi}$
 w^{1118} ; $esg::Gal4$, $UAS::mCherry$, $UAS::GCaMP3$; $tub::Gal80^{ts}$, $Su(H)Gbe::Gal80/UAS::Stim^{OE}$,
 $UAS::Orai^{OE}$
 w^{1118} ; $esg::Gal4$, $UAS::mCherry$, $UAS::GCaMP3$; $tub::Gal80^{ts}$, $Su(H)Gbe::Gal80/UAS::IP3R^{OE}$

Fig. 3b

w^{1118} ; $esg::Gal4$, $UAS::nlsGFP$, $tub::Gal80^{ts}/+$
 w^{1118} ; $esg::Gal4$, $UAS::nlsGFP$, $tub::Gal80^{ts}/UAS::Serca^{RNAi}$
 w^{1118} ; $esg::Gal4$, $UAS::nlsGFP$, $tub::Gal80^{ts}/UAS::Stim^{RNAi}$

$w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts} / UAS::Stim^{RNAi}$; $UAS::Serca^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts} / UAS::Stim^{OE}$, $UAS::Orai^{OE}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts} / UAS::Pmca^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts} / UAS::IP3R^{OE}$

Fig.3c

$w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts} / +$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts} / UAS::Serca^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts} / UAS::Stim^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts} / UAS::Stim^{RNAi}; UAS::Serca^{RNAi}$

Fig.3d

$w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/Serca^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}; Crtc^{25-3}/UAS::Serca^{RNAi}; Crtc^{25-3}$

Fig.3e

$w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts} / +$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}; Crtc^{25-3}/Crtc^{25-3}$

Fig.3f

$w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts} / +$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}; UAS::CRTC^{OE} / +$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts} / UAS::mGluR^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}; UAS::CRTC^{OE} / UAS::mGluR^{RNAi}$

Fig.4a

$w^{1118}; esg::Gal4, UAS::eYFP, tub::Gal80^{ts}; Su(H)Gbe::Gal80 / +$
 $w^{1118}; esg::Gal4, UAS::eYFP, tub::Gal80^{ts}; Su(H)Gbe::Gal80 / UAS::IP3R^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::eYFP, tub::Gal80^{ts}; Su(H)Gbe::Gal80 / UAS::RAS^{V12}$
 $w^{1118}; esg::Gal4, UAS::eYFP, tub::Gal80^{ts}; Su(H)Gbe::Gal80 / UAS::IP3R^{RNAi}; UAS::RAS^{V12}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts} / +$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts} / UAS::IP3R^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts} / UAS::InR^{WT}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts} / UAS::IP3R^{RNAi}; UAS::InR^{WT}$

Fig.4b

$w^{1118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts}, Su(H)Gbe::Gal80 / +$
 $w^{1118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts},$
 $Su(H)Gbe::Gal80 / UAS::IP3R^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts}, Su(H)Gbe::Gal80 / UAS::RAS^{V12}$

$w^{1118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts},$
 $Su(H)Gbe::Gal80/UAS::IP3R^{RNAi}; UAS::RAS^{V12}$
 $w^{1118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts}, Su(H)Gbe::Gal80/UAS::InR^{WT}$
 $w^{1118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts},$
 $Su(H)Gbe::Gal80/UAS::IP3R^{RNAi}; UAS::InR^{WT}$

Fig.4c

$w^{1118}; esg::Gal4, UAS::eYFP; tub::Gal80^{ts}; Su(H)Gbe::Gal80/+$
 $w^{1118}; esg::Gal4, UAS::eYFP; tub::Gal80^{ts}; Su(H)Gbe::Gal80/UAS::Serca^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::eYFP; tub::Gal80^{ts}; Su(H)Gbe::Gal80/UAS::InR^{DN}$
 $w^{1118}; esg::Gal4, UAS::YFP; tub::Gal80^{ts}; Su(H)Gbe::Gal80/UAS::Serca^{RNAi}; UAS::InR^{DN}$
 $w^{1118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts}, Su(H)Gbe::Gal80/+$
 $w^{1118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts},$
 $Su(H)Gbe::Gal80/UAS::Serca^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts}, Su(H)Gbe::Gal80/UAS::InR^{DN}$
 $w^{1118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts}, Su(H)Gbe::Gal80/+$
 $UAS::Serca^{RNAi}; UAS::InR^{DN}$

Fig.4d

$w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/+$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::Pmca^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::InR^{DN}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}; UAS::CRTC^{OE}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::Pmca^{RNAi}; UAS::InR^{DN}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::CRTC^{OE}; UAS::InR^{DN}$

exFig.2a-e

$w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/+$
 $w^{1118}; esg::Gal4, UAS::eYFP; tub::Gal80^{ts}, Su(H)Gbe::Gal80/+$

exFig.2f-g

$w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}; UAS::FLP, tubFRT-CD2-FRTGal4/+$

exFig.2h

$hsflp^{112}; tubGAL4, UAS::CD8GFP; FRT82B, tub::Gal80/FRT82B$

exFig.3c, d, f

$w^{1118}; NP1::Gal4, tub::Gal80^{ts}/+$
 $w^{1118}; NP1::Gal4, tub::Gal80^{ts}/UAS::Eaat1^{RNAi}$

exFig.3e

$w^{1118}; NP1::Gal4, tub::Gal80^{ts}/+$
 $w^{1118}; NP1::Gal4, tub::Gal80^{ts}/UAS::Eaat1^{OE}$

exFig.3g

$w^{1118}; NP1::Gal4, tub::Gal80^{ts}; 2xSTAT::GFP/+$
 $w^{1118}; NP1::Gal4, tub::Gal80^{ts}; 2xSTAT::GFP/UAS::Eaat1^{RNAi}$

exFig.4b,4d

$w^{1118}; vGlut^{CNS}::Gal4, UAS::mCD8GFP/+$

exFig.4e

$w^{1118}; act::Gal4, UAS::GCaMP3/+$
 $w^{1118}; act::Gal4, UAS::mCD8GFP/+$

exFig.4f

$w^{1118}; esg::Gal4, tub::Gal80^{ts}, UAS::mCherry, UAS::GCaMP3/Su(H)Gbe::LacZ$

exFig.5a-d

$w^{1118}; esg::Gal4, tub::Gal80^{ts}; Su(H)Gbe::Gal80/UAS::tdTomato-2A-GCaMP5$
 $w^{1118}; esg::Gal4, UAS::GCaMP3, UAS::mCherry; tub::Gal80^{ts}, Su(H)Gbe::Gal80/+$
 $w^{1118}; esg::Gal4; tub::Gal80^{ts}; Su(H)Gbe::Gal80/UAS::IVS-GCaMP6s$

exFig.6a-c

$w^{1118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts}, Su(H)Gbe::Gal80/+$
 $w^{1118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts},$
 $Su(H)Gbe::Gal80/UAS::mGluR^{RNAi}$

exFig.6d-e

$w^{1118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts}, Su(H)Gbe::Gal80/+$
 $w^{1118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts}, Su(H)Gbe::Gal80/UAS::GaQ^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts},$
 $Su(H)Gbe::Gal80/UAS::Gbta^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts},$
 $Su(H)Gbe::Gal80/UAS::PlcBeta^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts}, Su(H)Gbe::Gal80/UAS::GaO^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts}, Su(H)Gbe::Gal80/UAS::PTX$
 $w^{1118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts}, Su(H)Gbe::Gal80/UAS::Gal^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts}, Su(H)Gbe::Gal80/UAS::GaS^{RNAi}$

exFig.6f

$w^{1118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts}, Su(H)Gbe::Gal80/+$

$w^{118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts},$
 $Su(H)Gbe::Gal80/UAS::CavBeta^{RNAi}$
 $w^{118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts}, Su(H)Gbe::Gal80/UAS::Cac^{RNAi}$
 $w^{118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts},$
 $Su(H)Gbe::Gal80/UAS::NacChBac$
 $w^{118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts},$
 $Su(H)Gbe::Gal80/UAS::DeltaORK$

exFig.7b

$w^{118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/+$
 $w^{118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::Sercap^{RNAi}$
 $w^{118}; Su(H)Gbe::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/+$
 $w^{118}; Su(H)Gbe::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::Sercap^{RNAi}$
 $w^{118}; esg::Gal4, UAS::eYFP, tub::Gal80^{ts}; Su(H)Gbe::Gal80/+$
 $w^{118}; esg::Gal4, UAS::eYFP, tub::Gal80^{ts}; Su(H)Gbe::Gal80/UAS::Sercap^{RNAi}$

exFig.7c

$hsflp^{112}; tub::Gal80FRT40A; tubGAL4, UAS::CD8GFP/FRT40A$
 $hsflp^{112}; tub::Gal80FRT40A; tubGAL4, UAS::CD8GFP/FRT40A; UAS::Sercap^{RNAi}$
 $hsflp^{112}; tub::Gal80FRT40A; tubGAL4, UAS::CD8GFP/FRT40A; UAS::Stim^{RNAi}$
 $hsflp^{112}; tub::Gal80FRT40A; tubGAL4, UAS::CD8GFP/FRT40A; UAS::Pmcap^{RNAi}$

exFig.7d

$hsflp^{112}; tub::Gal80FRT42D; tubGAL4, UAS::CD8GFP/FRT42D$
 $hsflp^{112}; tub::Gal80FRT42D; tubGAL4, UAS::CD8GFP/FRT42D, Sercap^{Kum170}$

exFig.7e

$hsflp^{112}, FRT19A, tub::Gal80; tub::Gal4, UAS::GFP/FRT19A$
 $hsflp^{112}, FRT19A, tub::Gal80; tub::Gal4, UAS::GFP/FRT19A, Sim^A$

exFig.7f

$w^{118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/+$
 $w^{118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::Sercap^{RNAi}$
 $w^{118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::Orai, UAS::Stim$
 $w^{118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::Pmcap^{RNAi}$
 $w^{118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::Stim^{RNAi}$
 $w^{118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::Sercap^{RNAi}, UAS::Stim^{RNAi}$

exFig.8b-e

$w^{118}/Su(H)Gbe::LacZ; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/+$
 $w^{118}/Su(H)Gbe::LacZ; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::Pmcap^{RNAi}$
 $w^{118}/Su(H)Gbe::LacZ; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::Sercap^{RNAi}$

$w^{1118}/Su(H)Gbe::LacZ; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::Notch^{RNAi}$
 $w^{1118}/Su(H)Gbe::LacZ; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::Crtc^{OE}$
 $w^{1118}/Su(H)Gbe::LacZ; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::Serca^{RNAi}; UAS::Stim^{RNAi}$

exFig.8f

$w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/+$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::Serca^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::Orai, UAS::Stim$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::Pmca^{RNAi}$

exFig.8g-h

$hsflp^{112}; tub::Gal80, FRT40A; tubGAL4, UAS::CD8GFP/FRT40A$
 $hsflp^{112}; tub::Gal80, FRT40A; tubGAL4, UAS::CD8GFP/UAS::Serca^{RNAi}; FRT40A$
 $hsflp^{112}; tub::Gal80, FRT40A; tubGAL4, UAS::CD8GFP/FRT40A; UAS::Serca^{RNAi}$
 $hsflp^{112}; tub::Gal80, FRT40A; tubGAL4, UAS::CD8GFP/FRT40A; UAS::Pmca^{RNAi}$

exFig.8i

$w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/+$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::Notch^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::CanB2^{RNAi}/^+$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::CanB2^{RNAi}/UAS::Notch^{RNAi}$

exFig.8j

$w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/+$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::NICD$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::Serca^{RNAi}/^+$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::Serca^{RNAi}/UAS::NICD$

exFig.9a

$w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/+$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::Serca^{RNAi}/^+$

exFig.9b

$w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/^+$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::Serca^{RNAi}/^+$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::XBPI^s$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::Serca^{RNAi}/UAS::XBPI^s$

exFig.9c

$w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/^+$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::Serca^{RNAi}$

$w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::Crtc^{OE}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::Pmca^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::InR^{WT}$

exFig.9d

$w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/+$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::CaMKI^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::CaMKII^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::CanB2^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::CanA1^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}, UAS::Serca^{RNAi}/+$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}, UAS::Serca^{RNAi}/UAS::CaMKI^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}, UAS::Serca^{RNAi}/UAS::CaMKII^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}, UAS::Serca^{RNAi}/UAS::CanB2^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}, UAS::Serca^{RNAi}/UAS::CanA1^{RNAi}$

exFig.9e

$w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/+$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::Pmca^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::Stim, UAS::Orai$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}, UAS::CanB2^{RNAi}/+$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}, UAS::CanB2^{RNAi}/UAS::Pmca^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}, UAS::CanB2^{RNAi}/UAS::Stim, UAS::Orai$

exFig.9f

$w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/+$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::CanA14F^{ACT}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::Pp2B14D^{ACT}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::CanA1^{ACT}$

exFig.9g

$hsflp^{112}; tub::Gal80FRT42D; tubGAL4, UAS::CD8GFP/FRT42D$
 $hsflp^{112}; tub::Gal80FRT42D; tubGAL4, UAS::CD8GFP/FRT42D, CanB2^{KO}$
 $hsflp^{112}; tub::Gal80FRT40A; tubGAL4, UAS::CD8GFP/FRT40A$
 $hsflp^{112}; tub::Gal80FRT40A; tubGAL4, UAS::CD8GFP/FRT40; UAS::CanB2^{RNAi}$
 $hsflp^{112}; tubGAL4, UAS::CD8GFP; tub::Gal80, FRT82B/FRT82B$
 $hsflp^{112}; tubGAL4, UAS::CD8GFP; tub::Gal80, FRT82B/UAS::CanA14F^{ACT}; FRT82B$

exFig.9h

$w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/+$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::Pmca^{RNAi}$

$w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::Pp2B14D^{ACT}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}; Crtc^{25-3}/Crtc^{25-3}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}; Crtc^{25-3}/UAS::Pmca^{RNAi}; Crtc^{25-3}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}; Crtc^{25-3}/UAS::Pp2B14D^{ACT}; Crtc^{25-3}$

exFig.9i

$w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/+$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::CRTC::HA$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::CRTC::SA-HA$

exFig.9j

$w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/+$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::CRTC::HA$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}; Crtc^{25-3}/Crtc^{25-3}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}; Crtc^{25-3}/UAS::Sercd^{RNAi}; Crtc^{25-3}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::Pp2B14D^{ACT}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}; Crtc^{25-3}/UAS::Pp2B14D^{ACT}; Crtc^{25-3}$

exFig.9k

$w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/+$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::CRTC::HA/+$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::CRTC::HA/UAS::Gaq^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::CRTC::HA/UAS::IP3R^{RNAi}$

exFig.9l

$w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/+$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::CREB^{OE}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::CREB^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::CBP^{RNAi}$

exFig.9m

$w^{1118}; NP1::Gal4, tub::Gal80^{ts}/+$
 $w^{1118}; NP1::Gal4, tub::Gal80^{ts}/UAS::Pmca^{RNAi}$
 $w^{1118}; NP1::Gal4, tub::Gal80^{ts}/UAS::CanB2^{RNAi}$
 $w^{1118}; Elav::Gal4, tub::Gal80^{ts}/+$
 $w^{1118}; Elav::Gal4, tub::Gal80^{ts}/UAS::mGluR^{RNAi}$

exFig.10b-c

$w^{1118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts}, Su(H)Gbe::Gal80/+$
 $w^{1118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts},$
 $Su(H)Gbe::Gal80/UAS::Notch^{RNAi}$

$w^{1118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts}, Su(H)Gbe::Gal80/UAS::Upd2$
 $w^{1118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts}, Su(H)Gbe::Gal80/UAS::Ras^{V12}$
 $w^{1118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts}, Su(H)Gbe::Gal80/UAS::InR^{WT}$
 $w^{1118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts}, Su(H)Gbe::Gal80/UAS::InR^{DN}$
 $w^{1118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts}, Su(H)Gbe::Gal80/UAS::CncC$

exFig.10d

$w^{1118}; esg::Gal4, tub::Gal80^{ts}; Su(H)Gbe::Gal80/UAS::tdTomato-2A-GCaMP5$

exFig.10f-g

$w^{1118}; esg::Gal4, UAS::eYFP; tub::Gal80^{ts}, Su(H)Gbe::Gal80/+$
 $w^{1118}; esg::Gal4, UAS::eYFP; tub::Gal80^{ts}, Su(H)Gbe::Gal80/UAS::InR^{DN}$
 $w^{1118}; esg::Gal4, UAS::eYFP; tub::Gal80^{ts}, Su(H)Gbe::Gal80/UAS::IP3R^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::eYFP; tub::Gal80^{ts}, Su(H)Gbe::Gal80/UAS::Orai^{RNAi}$

exFig.10h

$w^{1118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts}, Su(H)Gbe::Gal80/+$
 $w^{1118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts}, Su(H)Gbe::Gal80/UAS::IP3R^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts}, Su(H)Gbe::Gal80/UAS::Orai^{RNAi}$

exFig.10i

$w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/+$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::CanB2^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}; Crtc^{25-3}/Crtc^{25-3}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::mGluR^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::IP3R^{RNAi}$

exFig.10j

$w^{1118}; esg::Gal4, UAS::mCD8GFP, tub::Gal80^{ts}; UAS::InR^{WT}/+$
 $w^{1118}; esg::Gal4, UAS::mCD8GFP, tub::Gal80^{ts}; UAS::InR^{WT}/UAS::Crtc^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}; Crtc^{25-3}/UAS::InR^{WT}, Crtc^{25-3}$
 $w^{1118}; esg::Gal4, UAS::mCD8GFP, tub::Gal80^{ts}; UAS::InR^{WT}/UAS::Stim^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::mCD8GFP, tub::Gal80^{ts}; UAS::InR^{WT}/UAS::CanB2^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::mCD8GFP, tub::Gal80^{ts}; UAS::Hep^{WT}/+$
 $w^{1118}; esg::Gal4, UAS::mCD8GFP, tub::Gal80^{ts} /+$
 $w^{1118}; esg::Gal4, UAS::mCD8GFP, tub::Gal80^{ts}/UAS::IP3R^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::mCD8GFP, tub::Gal80^{ts}; UAS::Hep^{WT}/UAS::IP3R^{RNAi}$

exFig.10k

$w^{1118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts}, Su(H)Gbe::Gal80/+$

$w^{1118}; esg::Gal4, UAS::mCherry, UAS::GCaMP3; tub::Gal80^{ts},$
 $Su(H)Gbe::Gal80/UAS::mGluR^{RNAi}$

exFig.10l

$w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/+$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::Hop^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}; UAS::Pmca^{RNAi}/+$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}; UAS::Pmca^{RNAi}/UAS::Hop^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::Dome^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}; UAS::Serca^{RNAi}/+$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}; UAS::Serca^{RNAi}/UAS::Hop^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}; UAS::Serca^{RNAi}/UAS::Dome^{RNAi}$

exFig.10m

$w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/+$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}/UAS::fos^{RNAi}$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}; UAS::Serca^{RNAi}/+$
 $w^{1118}; esg::Gal4, UAS::nlsGFP, tub::Gal80^{ts}; UAS::Serca^{RNAi}/UAS::fos^{RNAi}$